STUDIES ON SYNTHETIC MEDIUMS

II. SUGAR FERMENTATIONS IN SYNTHETIC MEDIUMS

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It was found in the experiments already reported[1] that the members of the colon typhoid group grew very well on a synthetic medium of very simple chemical composition, and that after growing on this medium for a month still retained their sugar fermenting properties intact. The next step in the investigation then was to add the sugar directly to the synthetic medium, and grow the organisms on this.

The medium, the same as previously used, consisted of the following:

129.5	c c of molar	H_3PO_4
18.8	c c of molar	CH_3COOH
17.8	c c of molar	NH_4OH
100	c c of molar	NaOH
100	c c of molar	KOH
10	c c of 0.01%	Fe_2Cl_6
10	c c of 0.01%	$MgSO_4$
10	c c of 0.01%	$CaCl_2$

diluted up to give 1 liter of medium.

Using this as a basis the 4 sugars used were added in amounts sufficient to give 1% sugar. The 4 sugars were dextrose, saccharose, lactose and mannite. About 5-7 c c of medium was placed in a test tube containing a small capsule, after the tube had been sterilized. The medium had first had added in 1 case litmus as an indicator and in the other Andrade's indicator.[2] The medium was sterilized by steam on 3 successive days. It was sterilized for exactly 15 minutes from the time the burner of the sterilizer was lighted. exactly 15 minutes from the time the burner of the sterilizer was lighted. A medium of this composition has an initial hydrogen-ion concentration of 10^{-7n}.

The cultures used were 24-hour transplants, the synthetic medium being inoculated with a 1 mm. loopful of the culture. Four sets of each culture were planted at once: (1) synthetic containing Andrade's indicator; (2) synthetic containing litmus; (3) synthetic containing no indicator, to be tested for the hydrogen-ion concentration, and (4) sugar broth containing litmus. The cultures and controls of each kind of medium were incubated for 24 hours and readings as to the formation of acid and gas. Like readings were taken at 48 hours and 8 days.

The hydrogen-ion concentration was determined in the set of cultures containing no indicator. The tubes were filled so that each tube contained not less than 10 c c, as the hydrogen-ion concentration had to be determined at the end of 24 hours, which used up about one-half the culture and the other half was incubated for 8 days and the final hydrogen-ion concentration determined then. The hydrogen-ion concentration was determined by matching

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[1] Jour. Infect. Dis., 1919, 24, p. 9.

[2] Holman Jour. Infect. Dis., 1914, 15, p. 227.

against a series of phosphate standards made according to Sörensen using the Clark and Lub[8] indicators together with methyl-red, methyl orange and phenolphthalein, and in no case was a hydrogen-ion concentration determined with the use of less than 3 indicators. In some cases when the indicators did not overlap sufficiently it was necessary to estimate, and this was the case in all cases recorded as $10^{-6.5n}$.

The litmus used was made from Merck's cube litmus and on the whole was unsatisfactory as it reduced very readily. The color could sometimes be restored by shaking. From this standpoint the Andrade's indicator was much more satisfactory. In the case of dextrose, only 3 of the 60 organisms tried failed to produce acid or gas when they did so in broth. In the few cases in which the organism took longer to reach the end reaction on the synthetic medium than it did on broth, the failure may have been due to the fact that the organism had been for a long time on agar and no effort was made to rejuvenate the organism before using, transplants being made directly from the agar to the synthetic sugar medium. This may also explain some of the failures on the broth where gas and acid formation were expected, but failed to appear. The difference of 24 hours in the formation of gas in the synthetic medium containing litmus and that containing Andrade's indicator has little significance, as one set was planted in the morning and the other in the afternoon, and the next day they were examined as quickly as possible and in cases in which only a few tiny bubbles of gas had formed they were recorded as simply forming acid.

The formation of acid and gas in saccharose coincided in the sugar broth and the sugar synthetic medium, except that in a few cases the formation was somewhat slower in the second. The same statement may be made of the organisms planted on the lactose and mannite synthetic medium. In the case of lactose there were 2 organisms that worked more quickly on the synthetic medium than on broth, while with the mannite 2 organisms produced gas in the synthetic medium that only produced acid in the broth.

It can readily be seen that a simple sugar medium like this has distinct advantages over a sugar broth, as it does away with the necessity of first freeing the meat infusion of sugar. This medium could be of special advantage in routine field work, as the materials can be carried in less space and the medium is much more easily made, since it could readily be put up in tablet form, and by dissolving these tablets in sterile distilled water a sterile medium could be obtained at once.

[8] Jour. Bacteriol., 1917, 2, p. 1.